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# Effect of Crude Oil Polluted Soil and Substrate Quantity on Some Morphological Characters of *Pleurotus ostreatus* (Jacq.) P. Kumm and *Pleurotus pulmonarius* (Fries) Quel Fruit Bodies

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**Abstract**

The study was conducted to determine the influence of different levels of crude oil pollution on some morphological characteristics of *P. ostreatus* and *P. pulmonarius* fruit bodies. Crude oil treatment was done at 2%, 4% and 6% levels to 2,500 g/kg of garden soil. *A. gayanus* substrate was used in two quantity layers of 150 g/kg (4 cm) and 300 g/kg (8 cm) and placed on top of the polluted soil before spawn inoculation. The Cap Size (C.S cm), Stipe Length (S.L cm) and Weight (Wt. g/kg) of each mature mushroom fruit body were determined. Data collected were analysed using Analysis of Variance (ANOVA), while mean separation was done using Duncan Multiple Range Test (DMRT). Results showed that mean and standard error mean (mean± SEM) of C.S, S.L, and Wt of fruit bodies of both oyster mushrooms were not significantly different at $p<0.05$. Therefore, the various levels of crude oil pollution (2%, 4%, and 6%), as well as *A. gayanus* substrate layers/quantities of 4 cm/150 g/kg and 8 cm/300 g/kg did not affect some of the morphological characters of the Oyster mushroom species studied.

**Keywords:** Mushroom; Crude oil; Fungi; Polluted soil

## Introduction

The ability of mushrooms to utilize various lignocellulosic substrates, makes their cultivation possible in different parts of the world [1]. Substrate type is one of the major factors affecting the yield and quality of oyster mushroom [2]. A substrate in mushroom cultivation may be defined as a kind of lingo cellulose material which supports the growth, development and fruiting of mushroom [3]. Most of all edible mushroom species can utilize various kinds of substrate materials depending on their availability in different places. The nutrient composition of these substrate materials is one of the factors affecting the saprobiotic colonization of the mycelia of cultivated mushrooms [4].

The growth, quantity and quality of yield of the desired mushrooms depend on the utilization of nutrients and physiochemical environment in the medium or substrate. Therefore, the growth of diverse type of mushrooms requires different type of substrates and availability of varied type of materials may dictate which type is used. Kumar et al. reported the successful cultivation of oyster mushroom on conventional substrates sufficiently available, which are not utilized properly and productively.

## Mushrooms and Crude Oil

Several fungi like *Pleurotus ostreatus, Lentinus subnudus* and *Pleurotus tuber-regium* have been grown in crude oil mediated substrate [5-7]. Ogbo and Okhuoya [7] showed that *P. tuberregium* was able to decontaminate crude oil contaminated soils reducing the various petroleum hydrocarbons in crude oil to varying degrees. The contaminants instead of inhibiting the growth of the fungus, enhanced it. The ability of white rot fungi to degrade petroleum hydrocarbons can be due to the fact that these fungi are uniquely equipped as soil remediation agents [8]. White rot fungi are filamentous organisms and as such have the propensity to extend through soil in search of new substrates to exploit and thus colonize larger surface area than bacteria. Furthermore, with the aid of their highly oxidative lignin degrading systems, they are able to oxidize extremely hydrophobic substrates [9]. The only drawback here is that these environmental pollutants or their degradation products may inhibit their lignin degrading system [10,11]. Petroleum hydrocarbons have been shown to improve the growth of white rot fungi in contaminated soils by increasing their cap diameter, stipe length and yield indicating that the contaminants have a fertilizer effect [5,12].

This investigation aims to explore the influence of crude oil pollutant and substrate quantity on some morphological characteristics of *P. ostreatus* and *P. pulmonarius* fruit bodies studied.

## Materials and Methods

### Study area

The study was conducted in the screen house of the Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located between longitude 7° and 70°05°E and latitude 5° and 5°25°N; with humid tropical climate. Rainfall is bi-modally distributed with peaks between July and September of each year. Annual rainfall is approximately 170 mm, spread between April and November each year.

### Source of culture and spawn multiplication

Pure mycelia culture of *P. ostreatus* and *P. pulmonarius* were obtained and multiplied at the department of biotechnology, Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos State. Spawns were produced using sorghum grains. Sorghum grains were washed and soaked in tap water overnight. Grains were further boiled in same tap water in the ratio of 1:1 (sorghum grain: water) for 15 mins, using the industrial cooking gas as a local heat source. 4% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$ were added to optimize pH and prevent clumping of grains respectively as described by Muhammad et al. [13]. Completely drained Sorghum grains were stuffed in glass Bama bottles tightly sealed with Aluminium foil and before being sterilized in an autoclave at 121°C for 30 mins. After sterilization, the bottles were allowed to cool, before they were inoculated with actively growing mycelia of *P. ostreatus* and *P. pulmonarius* by grain-to- grain transfer and incubated in the dark at (27 ± 2°C) for 10-15 days until the grains were fully colonized by mycelia [14].

### Source of pollutant (Crude oil)

The Bonny Light crude oil was obtained from the Nigerian National Petroleum Co-operation. (N.N.P.C.), Port Harcourt.

### Bucket preparation

Five-liter transparent plastic buckets were used during this investigation. The upper half of each 5-liter plastic bucket was perforated, with two lateral holes of 5 mm diameter Okulehie and Okwujiako [15].

### Source and soil preparation

Loamy soil sample was collected from a farmland within the vicinity of Government College Umuahia, Abia State. Soil sample was collected from the 'A' horizon (0-25 cm). To obtain a semi-sterile soil, the soil was treated in three successive pasteurization periods of 2 hrs each, for two consecutive days, following the modified method of Kristanti et al. [16]. The moisture content of the soil was adjusted to 60% of maximum water-holding capacity (WHC; 0.2 ml/g dry soil) after each pasteurization.

### Crude oil pollution

Each perforated plastic bucket contained 2.5 kg of pasteurized soil polluted with 50 g, 100 g and 150 g w/v of crude oil to make 2%, 4% and 6% crude oil pollution of the soil.

### Source and preparation of substrate

*Andropogon gayanus*, a locally available straw substrate was obtained from a farmland in Umudike. The grass was dried and chopped into about 1-2 cm lengths before steeping it in tap water overnight to ensure adequate moisture content [17].

The soaked substrate was drained of excess water before being transferred into a metallic drum for pasteurization at 80°C for 2 hrs and was allowed to cool overnight as recommended by Muhammad et al. [13].

### Experimental procedure

The experiment was conducted using 2.5 lit plastic bowels. Three levels of crude oil pollution at 50 g, 100 g and 150 g w/v was used to homogenizes each 2500 g of the prepared soil sample to make 2%, 4% and 6% respectively. The various levels of crude oil polluted soil were poured into each perforated transparent plastic bowel while the control was not polluted with crude oil. All the crude oil treatment levels including control were made up of two groups. The first group consist of 4 cm (150 g) thick layer of prepared *A. gayanus* substrate placed on the surface of the crude oil polluted soil and inoculated with 30 g of grain based spawn of actively growing mycelia of *P. ostreatus* and *P. pulmonarius* while the second has 8 cm (300 g) thick layer of same substrate and was inoculated with 60 g grain based spawn of same mushrooms according to a modified method of Okwulehie and Okwujiako [15].

The spawn-inoculated substrate served as a mycelia mat on the surface of the crude oil polluted soil, contained in the buckets [18]. After spawn inoculation, spawn run was completed in the dark by covering the inoculated buckets with thick non-transparent polythene mat, until the substrate is fully colonized by the mycelia. Priormodial initiation was preceded by fruit body maturity before they were finally harvested.

### Determination of morphological

Some morphological characteristics of fruit bodies produced on each experimental unit of various percentage crude oil pollution and the control were recorded as follows.

**Cap diameter:** This was determined by placing a transparent ruler across the center of the pileus of each harvested mushroom fruit body and recording the value.

**Length of stipe:** This was determined by placing transparent ruler along the length of each fruit body stipe.

**Weight of fresh fruit bodies:** This was determined by weighing each fresh fruit body immediately after harvesting, using a portable digital balance.

### Statistical analysis

All the data collected from various samples were subjected to Analysis of Variance (ANOVA) while comparison between different means were done using Duncan multiple range test (DMRT) at $p<0.05$ level significance.

## Results and Discussion

| Mushrooms | | Level of crude oil pollution (%) | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| *P. ostreatus* | 4.99 ± 0.25b | 4.76 ± 0.21 b | 4.54 ± 1.10 b | 4.50 ± 1.46 b |

| | | | | |
|---|---|---|---|---|
| *P. pulmonarius* | 5.88 ± 0.35a | 5.65 ± 1.27 a | 5.59 ± 0.35 a | 5.31 ± 0.13 a |

**Table 1:** Effect of crude oil pollution and 150 g/4 cm *A. gayanus* substrate on the cap diameter of *P. ostreatus* and *P. pulmonarius* fruit bodies. Values are means of 3 replicates. Means with the same superscript are not significantly different p<0.05.

| **Mushrooms** | | **Level of crude oil pollution (%)** | | |
|---|---|---|---|---|
| | **0** | **2** | **4** | **6** |
| *P. ostreatus* | 2.32 ± 0.94 b | 2.35 ± 1.07 b | 2.84 ± 0.45 a | 2.42 ± 0.21 a |
| *P. pulmonarius* | 3.34 ± 0.31 a | 3.92 ± 1.05 a | 2.27 ± 0.29 b | 2.10 ± 0.21 b |

**Table 2:** Effect of crude oil pollution and 150 g/4 cm *A. gayanus* substrate on the stripe length of *P. ostreatus* and *P. pulmonarius* fruit bodies. Values are means of 3 replicates; means with the same superscript are not significantly different p<0.05.

| **Mushrooms** | | **Level of crude oil pollution (%)** | | |
|---|---|---|---|---|
| | **0** | **2** | **4** | **6** |
| *P. ostreatus* | 4.96 ± 0.39 a | 4.55 ± 0.32 b | 4.05 ± 1.77 a | 3.65 ± 0.61 a |
| *P. pulmonarius* | 4.68 ± 0.55 b | 4.63 ± 1.75 a | 4.13 ± 0.30 a | 4.00 ± 0.30 b |

**Table 3:** Effect of crude oil pollution and 150 g/4 cm *A. gayanus* substrate on the fresh fruit body weight of *P. ostreatus* and *P. pulmonarius*. Values are means of 3 replicates means with the same superscript are not significantly different p<0.05.

| **Mushrooms** | | **Level of crude oil pollution (%)** | | |
|---|---|---|---|---|
| | **0** | **2** | **4** | **6** |
| *P. ostreatus* | 4.32± 0.31 b | 4.55 ±0.11 b | 4.22 ±0.30 b | 4.55± 0.44 a |
| *P. pulmonarius* | 5.91± 0.32 a | 5.32± 0.34 a | 5.20± 0.35 a | 4.48± 0.51 a |

**Table 4:** Effect of crude oil pollution and 300 g/8 cm *A. gayanus* substrate on the cap diameter of *P. ostreatus* and *P. pulmonarius* fruit bodies. Values are means of 3 replicates. Means with the same superscript are not significantly different p<0.05

| **Mushrooms** | | **Level of crude oil pollution (%)** | | |
|---|---|---|---|---|
| | **0** | **2** | **4** | **6** |
| P. ostreatus | 2.44 ± 0.68 b | 2.31 ± 0.07 b | 2.11 ± 0.10 b | 2.26 ± 0.20 a |
| P. pulmonarius | 2.52 ± 0.63 a | 2.41 ± 0.25 a | 2.22 ± 0.20 a | 2.00 ± 0.54 b |

**Table 5:** Effect of crude oil pollution and 300 g/8 cm *A. gayanus* substrate on the stipe length of *P. ostreatus* and *P. pulmonarius* fruit bodies. Values are means of 3 replicates. Means with the same superscript are not significantly different p<0.05.

| **Mushrooms** | | **Level of crude oil pollution (%)** | | |
|---|---|---|---|---|
| | **0** | **2** | **4** | **6** |
| *P. ostreatus* | 3.70 ± 0.20 b | 2.88 ± 0.14 b | 4.95 ± 0.55 a | 3.19 ± 0.25 a |
| *P. pulmonarius* | 5.22 ± 0.76 a | 4.99 ± 0.61 a | 4.30 ± 0.60 b | 4.01 ± 0.92 a |

**Table 6:** Effect of crude pollution and 3000 g/8 cm *A. gayanus* substrate on the fresh fruit body weight of *P. ostreatus* and *P. pulmonarius*. Values are means of 3 replicates. Means with the same superscript are not significantly different p<0.05.

## Discussion

In this work, effect of crude oil pollution and substrate quantity on some morphological characteristics of *P. ostreatus* and *P. pulmonarius* fruit bodies was successfully carried out. The results in Table 1 showed that both *P. ostreatus* and *P. pulmonarius* were able to grow on *A. gayanus* substrate of 150 g/4 cm substrate quantity, increase in crude oil pollution led to a gradual decrease in the cap diameter of fruit bodies of both mushrooms. This observation is not in isolation because Eggen and Vaclav [19] also pointed out that concentration of pollutant such as crude oil is one of the factors that leads to fungal growth efficiency in a medium or substrate. The result also reveals that *P. pulmonarius* produced fruit bodies with bigger cap diameter at all levels of crude oil pollution. This could be as a result of certain inherent or genetic factors in the mushroom which gives it a more cellulose degrading ability due to secretion of certain enzymes like cellulose, lignase, peroxidise etc. [19-21]. Stipe length of the two mushrooms was also evaluated (Table 2). It was observed that increase in crude oil pollution at different levels (2-6%) caused a significant decrease on the stipe length of the fruit bodies of both mushroom species. Carbon is the major component of crude oil and is required by mushrooms during their vegetative stage [3,22]. During fruit body production (i.e., reproductive) stage of mushrooms, oxygen is required for effective fruitification and development of the cap and the stipe [23,24]. This could be the major reasons why increase in crude oil hindered the Morphological development of both fruit bodies [3,21,25]

Increase in crude oil pollution also caused a reduction in the fresh weight of fruit bodies of the two oyster mushroom species (Table 3), there was no significant different in the fresh fruit body weight of both mushrooms at various levels of crude oil pollution $p<0.05$. This observed reduction in fruit body weight with attendant increase in crude oil pollution is synonymous with the decrease in cap diameter and stipe length of the fruit bodies of both mushroom species. This shows that whatever that affects the size of fruit body also affects its weight and general yield across the substrate. This result was in-line with the works of Okwulehis and Okwujiaka [15], Assan and Mpofu [1] who reported that the unit weight of mushroom fruit bodies collected from any substrate determines its yield and Biological efficiency.

Table 4 showed the results of the cap diameter of *P. ostreatus* and *P. pulmonarius* fruit bodies harvested from 300 g/8 cm layer of *A. gayanus* substrate as affected by different levels of crude oil pollution. There was no significant difference at $p<0.05$ in the sizes of the cap of both oyster mushrooms at 150 g/4 cm (Table 1) and 300 g/8 cm. This shows that quantity of substrate has no significant effect on the size of the cap of both fruit bodies because cap sizes at higher substrate layer (300 g/8 cm) were similar to those of lower substrate layer (150 g/4 cm) (Table 1). This result was in consonance with the works of Assan et al. and Gitte et al. [26] who reported that increase in substrate quantity did not influence the days to fruiting, as well as size of individual fruit body, but may affect the yield and biological efficiency at the end of the fruiting cycle; since biological efficiency is calculated as percentage fresh fruit body yield in relation to the dry weight quantity of substrate [3,12,22].

Table 5 also reveals that substrate quantity and crude oil pollution did not significantly affect stipe length of both mushrooms at $p<0.05$. Unlike the results in Table 2 where *P. ostreatus* raised across the various levels of crude oil pollution at 300 g/8 cm level of substrate, stipe length of both oyster mushrooms was not significantly different. This could be that higher quantity of substrate is needed to reduce the negative effect of high concentration of crude oil pollution. Therefore, in a highly polluted environment, large quantity (layer) of substrate is needed to reduce the effect of the pollutant. This now brings solution to the problems observed by Eggen and Vaclav [21] stating that one of the factors influencing the effectiveness of fungal soil remediation is the concentration of the pollutant.

The result of the influence of 300 g/8 cm layer of *A. gayanus* substrate on the fruit body weight of *P. ostreatus* and *P. pulmonarius* was presented in Table 6. The result shows that fruit body weight of *P. pulmonarius* was clearly affected by crude oil pollution. Increase in crude oil pollution decreased fruit body weight from 2%-6%. A similar result was also observed in *P. ostreatus*, judging from 0-2%, but did not follow a regular trend at 4- 6%. The result observed in *P. ostreatus* could be due to other uncontrolled factors such as contaminants which affects mushroom cultivation [3,13,27]. These contaminants may have initially affected the experimental unit of the 4% crude oil pollution and those of 6% or vise versa.

## Conclusion

*Pleurotus ostreatus* and *P. pulmonarius* were successfully cultivated in a crude oil mediated *A. gayanus* straw substrate of 150 g/4 cm and 300 g/8 cm layers. A general consideration of the result showed that increase in crude oil pollution from 2- 6% affected the cap diameter, stipe length and fruit body weight of the two oyster mushrooms.

It was also observed that substrate quantity of 300 g/8 cm layer reduced the negative effect of crude on the morphological characteristics of both oyster mushrooms, but did not significantly affect their weight.

In any attempt to use *P. ostreatus* and *P. pulmonarius* to remediate a polluted soil, one should first determine the quantity or layer of substrate that should form adequate mycelia mat.

Larger quantity or layer of substrate should be used in a highly polluted environment; this will enhance mycelia colonization to overcome the negative influence of the pollutant on fruit body development.

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